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Hepatic Cryosurgery Precision: Evaluation of Ultrasonography, Thermometry, and Impedancemetry in a Pig Model

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One of the main problems of the use of liver cryosurgery is to be sure that a defined hepatic volume has been completely destroyed. We undertook an experimental pig study to determine histopathological evolution of cryolesions, to evaluate the value of intraoperative sonography, thermometry, and impedancemetry to monitor necrosis and to evaluate clinical and biological repercussions of hepatic cryosurgery. Forty-eight cryolesions were obtained by freezing each liver lobe of 12 experimental pigs during a 5-min contact with a flat cryoprobe cooled with liquid nitrogen. Cryolesions and the surrounding liver were monitored during cryosurgery by six thermocouple electrodes, five impedance electrodes, and intraoperative sonography. Animals were sacrificed immediately, 6 hr and between day 1 and day 32 after the procedure. Cryolesions were excised, and a full size pathological study was carried out. No morbidity or mortality was observed. At the end of the freezing time, cryolesions were hemispheric in shape, and their radius measured by sonography was 17.7 ± 1.2 mm (mean ±SD). Microscopic study showed sequential tissue alterations with edema, ischemic necrosis, tissue slough, and granulation. Cryolesions were sharply delineated from the normal liver tissue. The radius of necrosis at days 2 and 3 was 17 ± 0.3 mm (mean ±SD). It showed good correlation with the cryolesion size measured by intraoperative sonography. The temperature threshold to obtain complete normal liver necrosis was −15°C. We found impedancemetry too difficult to use and not precise enough to monitor cryonecrosis. We conclude that intraoperative sonography and thermometry are useful means to monitor the extent of cryonecrosis during liver cryosurgery.

KEY WORDS: **cryosurgery, instrumentation, temperature, impedance, ultrasonography, liver surgery**

INTRODUCTION

Liver metastases from colon and rectal cancers result in significant cancer related morbidity and mortality. Twenty-five to 30% of patients with an apparently complete colic resection for cancer will present with hepatic metastases [1]. Without any treatment, the half-life of patients with resectable hepatic metastases is 6–24 months [2]. Surgical hepatic resection is the current gold standard, with a 5-year survival of 23–40% [3,4], but only 10% of patients are eligible for surgery [5] because

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of a multiplicity of tumors involving both liver lobes, anatomic location of tumors close to major vessels, patients' comorbid conditions, or poor liver reserve (i.e., cirrhosis). Hepatic resection is indicated in patients with fewer than four metastases, allowing a resection margin wider than 1 cm [6]. Whenever hepatic resection is not possible, the only available treatments are systemic or locoregional chemotherapy, with a mean survival increase of 3 months [7].

Cryosurgery, i.e., destruction of tissues using deep subzero temperatures, has been used since 1907 for the treatment of cutaneous lesions [8]. Metallic instruments were dipped into liquid gas before application. This method was also used to treat cerebral tumors during craniotomy [9]. With this technique, the depth of freezing did not exceed 3 mm. Modern cryosurgery is based on automatic equipment, functioning with liquid nitrogen, displaying a continuously cooled cryoprobe [10].

Cryosurgery as a possible treatment of liver metastases or primary liver cancers has been reported [11–13]. Curiously, clinical applications seem to have preceded experimental evaluation of the method, and few experimental data on hepatic cryosurgery are available. The main problem is how to be sure that a defined hepatic volume has been completely destroyed following application of a cryoprobe. Goals of this experimental study were (1) definition of histopathological evolution of cryolesions induced in pig liver; (2) evaluation and comparison of intraoperative sonography, thermometry, and impedancemetry to monitor cryonecrosis; and (3) evaluation of clinical and biological repercussions of hepatic cryosurgery.

MATERIALS AND METHODS

Animals

Twelve pigs (weight 25–30 kg) were used. All the following procedures were performed in strict accordance with the National Institutes of Health (NIH) guidelines for care and use of laboratory animals (1985) and made under license of the French Ministry of Agriculture (Institut de Recherches Chirurgicales, UFR Alexis Carrel, Claude Bernard University, Lyon, France). Pigs were kept in a licensed laboratory piggery for a maximum of 6 weeks, and were normally fed.

Cryosurgical Equipment

Cryosurgical equipment (SMT KCH5 cryocauter) was supplied by Date-Medical (La Motte d'Aveillans, France). A liquid nitrogen reservoir was built into the handle and allowed use for 27 min. The cryoprobe was made of aluminum cooled internally with liquid nitrogen. A 20-mm-diameter extremity was used to perform contact cryosurgery.

Surgical Technique

Pigs were starved for 12 hr before the surgical procedure. After general anesthesia and intubation, the abdomen was antiseptically prepared with iodine polyvidone (Betadine). A midline incision was made and the liver exposed. Four cryolesions were performed in each animal, one on each liver lobe. They were placed far enough from the anterior liver edge in order to have a sufficient thickness of parenchyma, but not too close to the hepatic hilum or to the vena cava, to avoid major vascular or biliary injury. Thermocouples and impedance electrodes were fixed into the liver at precise distances from the cryoprobe (see below). After correct positioning of the cryoprobe at the liver surface, cryosurgery was applied for a 5-min period. Measurements were done during the 5 min of freezing and 20 min of thawing. After the four procedures were performed, laparotomy was closed and the animal was awakened.

Tissue Temperature Measurements

Six thermocouple electrodes with a 0.1-sec response time were inserted in the liver at 10, 15, 20, 25, 30, and 35 mm from the axis of the cryoprobe and at a 2-mm depth. They were connected to a temperature recorder (AM-7002, AnritsuMeter, Tokyo, Japan). It allowed us to perform a temperature measurement every second on each thermocouple needle. Data were then transmitted to a personal computer (LTE lite 4/25C, Compaq Computer Corp, Tokyo, Japan) for analysis of results and plotting (software Datacoll, Anritsu Meter Co, Tokyo, Japan). These measurements were performed for 16 cryolesions.

Tissue Impedance Measurements

Five impedance electrodes were inserted in the liver at 10, 12, 15, 17, and 20 mm from the axis of the cryoprobe and at a 2-mm depth. They were connected to an impedance recorder (Date Medical, La Motte d'Aveillans, France) that allowed a digital measurement every 15 sec. Data were then analyzed and plotted using a semilogarithmic scale. These measurements were performed for 16 cryolesions.

Intraoperative Ultrasound Monitoring

Continuous monitoring by intraoperative ultrasound was undertaken for 16 cryolesions. We accurately measured the size of the frozen region and controlled the advancing cold front. We used a small, 5 to 7.5-MHz, linear array ultrasound transducer (EUP-032T, Hitachi Medical Corp, Tokyo, Japan) connected to an echograph (EUB-415, Hitachi Medical Corp, Tokyo, Japan) and a SVHS recorder (Panasonic FS 100H, Matsushita Electric Industrial Co. Osaka, Japan). The transducer and the cryoprobe tip were placed on opposite sides of the liver lobe, so that the growing cold front moved away from the

cryoprobe and toward the transducer during the freezing period and away from the transducer during the thawing period. The entire freeze/thaw cycle was monitored continuously with ultrasound.

Biological Study

Blood samples were taken before and after cryosurgery and were repeated at the time of sacrifice of animals. Blood cell counts, hemoglobin, hematocrit, bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), lacticodehydrogenase (LDH) and γ-glutamyl transferase (γ-GT) levels were determined. Blood coagulation studies including platelet count, plasma fibrinogen, prothrombin time (PT) and activated partial thromboplastin time (PTT) were also performed.

Pathological Study

The pigs were sacrificed at varying intervals from immediately after cryosurgery to 32 days (immediate, 6 hr, days 1, 2, 3, 5, 7, 10, 15, 20, 25, 32) with an intravenous injection of pentobarbital. Liver was removed, cryolesions were measured, photographed, and sampled, cutting liver parenchyma at least at 2 cm from the lesion. Liver pieces were fixed in 10% formol (2.5 vol of formaldehyde at 37%, plus 0.5 vol of acetic acid at 100%, plus 7 vol of distilled water) and embedded in paraffin.

A special technique was used to obtain large slides (40 × 80 mm) featuring the entire cryolesion. Cryolesions were cut according to their main axis, 5-μm-thick slices were deparaffinized and stained with hematoxylin, phloxin, and safran for observation by optic microscopy. This technique allowed us to study the evolution of cryolesions, to measure precisely the cryolesion size and necrosis size and their evolution with time and to assess accurately the border between necrosis and normal surrounding liver.

RESULTS

Clinical and Biological Results

No mortality was encountered during and after cryosurgery. The pigs' central temperature never fell more than 1°C between the beginning of the first cryolesion and the end of the fourth. There was no major hemorrhage during the procedures. Occasionally we observed surface cracking after removal of the cryoprobe and minor hemorrhage during the thawing period. It was always easily controlled.

The animals appeared in good health during the follow-up period. There were no significant complications, including wound complication, infection, and peritonitis from bile leakage. At sacrifice, there was no intra-abdominal abscess. The only findings were major adhesions of cryolesions to surrounding structures.

Hemoglobin, hematocrit, and bilirubin remained unchanged. There was a major increase of AST, more than 10 times the normal value, whereas ALT had a minor increase of 1.5–2 times the normal value. Transaminases returned to normal within 1–2 weeks. Immediately after cryosurgery, there was an increase of polynuclear cells counts of 1.5–2.5 the normal value. They returned to normal within 1 week. We observed a minor alteration of blood coagulation studies during the first 24 hr after cryosurgery, i.e., a slight increase of PT and PTT.

Macroscopic Aspect of Cryolesions

During cryosurgery, cryolesions ("iceballs") were seen as white circular spreading zones. Liver parenchyma became hard and the cryoprobe was glued to the liver surface in a few seconds. After 5 min, the cryolesions did not spread any longer. A purple 2-mm wide circle was seen at its circumference. The cryolesion size, assessed by means of intraoperative ultrasound, was 17.7 ± 1.2 mm (mean radius ± SD). When freezing was stopped, one had to wait 15 sec to be able to remove the cryoprobe. The iceball shrank in 20 min, leaving behind a purple hemorrhagic zone of 23.2 ± 2.2 mm (mean radius ± SD). It was harder than the normal liver. Usually no blood was pearling from the thawed cryolesions.

At day 3, after removal of peritoneal adherence, cryolesions appeared as hemispheric black hard spots perfectly delineated from normal surrounding liver. From day 10, cryolesions became gray; they progressively reduced in depth and appeared at day 32 as a scar 20 mm in diameter and 3 mm thick, on the liver surface.

Tissue Temperature Measurements

Comparison of the 16 temperature curves showed they were reproducible. Figure 1 shows evolution of temperature monitoring for the six thermocouple electrodes during a whole freezing/thawing cycle. After 5 min of freezing, liver temperature reached −65°C close to the cryoprobe (10 mm from its axis). It decreased progressively from the axis of the cryoprobe to the periphery of the cryolesion, reaching −30°C at 15 mm and −5°C at 20 mm. At 25 mm, temperature never fell below 18°C and no major variation of animal temperature was observed.

Tissue Impedance Measurements

Figure 2 shows evolution of mean impedance for the five impedance electrodes during 16 freezing/thawing cycles (one measure every 15 sec) on a semilogarithmic plotting. There were considerable variations in measures for the 16 cryolesions and from one measure to another for the same cryolesions. The 1,000-kΩ plateau was reached at the 10- and 12-mm electrodes (distances from the axis of the cryoprobe) but was never reached for the 15-mm electrode. There was only minor variation in impedance for the 17- and 20-mm electrodes.

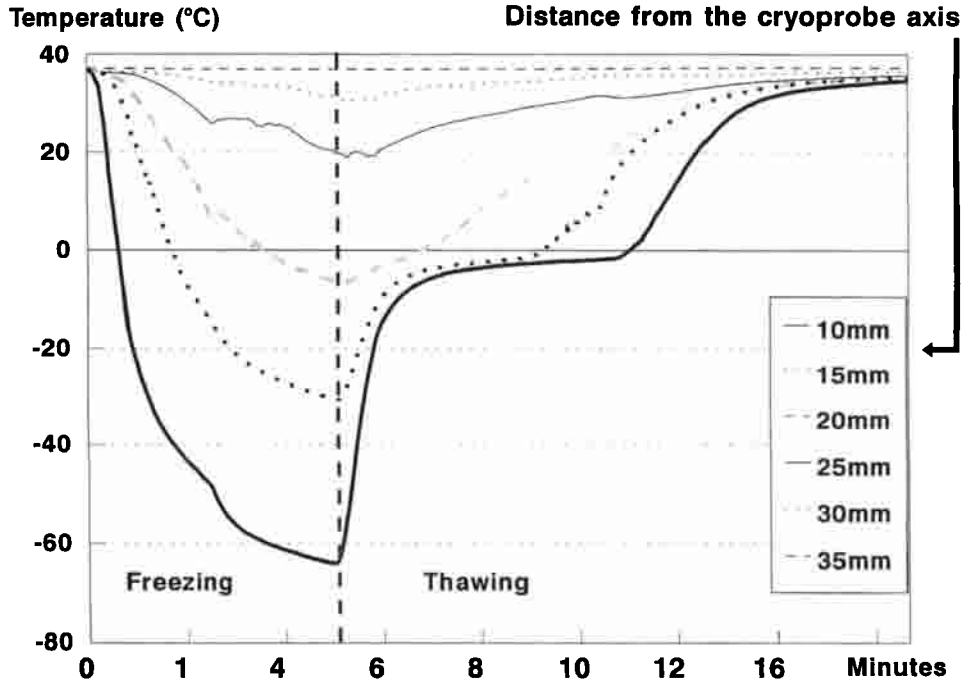

Fig. 1. Evolution of tissue temperature measurements during a whole freezing–thawing cycle (one measure every second on six thermocouple needles inserted in the liver at 10, 15, 20, 25, 30, and 35 mm from the axis of the cryoprobe and at a 2-mm depth). Thawing time has been limited to 13 min.

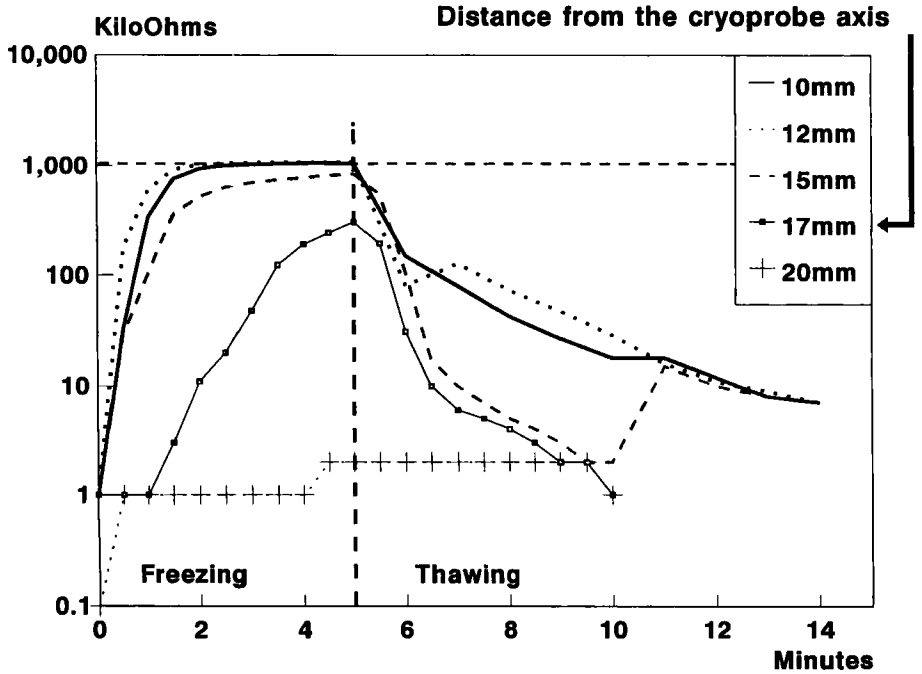

Fig. 2. Evolution (on a semilogarithmic plotting) of mean impedance for five impedance needles inserted in the liver at 10, 12, 15, 17, and 20 mm from the axis of the cryoprobe and at a 2 millimeter depth (16 freezing/thawing cycles, one measure every 15 sec). On this figure thawing time has been limited to 9 minutes.

Fig. 3. Macroscopic view of a cryolesion at day 3 showing the sharp delineation between cryonecrosis and normal liver (arrows).

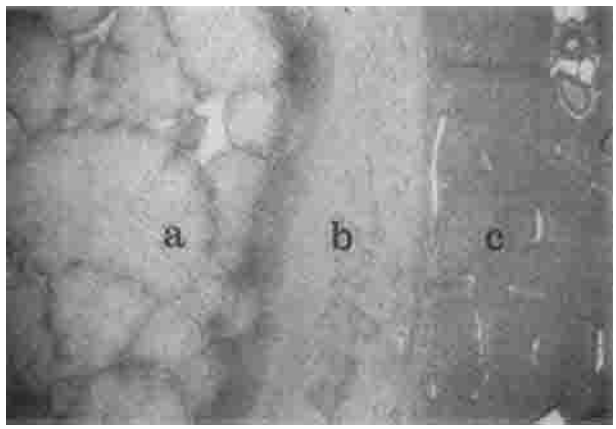

Fig. 4. Microscopic view of the cryolesion periphery at day 7 showing the necrotic area (**a**), leukocytic infiltration (**b**), and bile duct proliferation (**c**).

Intraoperative Ultrasound Monitoring

Within 15 sec of starting cryogen flow, a small, semicircular hyperechoic rim, bulging outward from the cryoprobe became visible on the ultrasound monitor. It represented the position of the solidification interface. It progressed into the liver parenchyma; at 5 min, it had achieved a radius of 17.7 ± 1.2 mm (mean ±SD). Acoustic shadowing by solidification interface and the volume of ice behind it had almost completely obscured the frozen hepatic surface as well as the cryoprobe tip. Evolution of iceball size and radius at 5 min of freezing were reproducible.

The frozen region began to thaw immediately after stopping cryogen flow. As thawing progressed, the hyperechoic rim receded toward the cryoprobe, leaving behind an hypoechoic zone. At full thaw the cryolesion appeared as a hypoechoic hemispherical region, easy to distinguish from the normal, surrounding liver.

Pathology Results

Microscopic examination of the cryolesion showed a distinct, sharply circumscribed area of coagulation necrosis. Immediately after freezing, the cryolesions could be easily demarcated from the normal surrounding liver but changes were minor. They consisted of a sinusoidal hyperemia in the whole of the treated area. Hepatocytes were more weakly stainable, and a beginning of glycogen disappearance could be observed with loss of cellular details. After 6 hr, a small zone with leukocytic interstitial infiltration became visible in the periphery of the lesion. There was a beginning fatty degeneration of hepatocytes. Lesions became more obvious with time. Degenerative changes with distinct circular areas of complete necrosis could be seen at days 2 and 3 (Fig. 3). Their mean size was 17 ± 0.3 mm (mean radius ± SD, 8 cryolesions). Until the seventh day, a dense leukocytic infiltration developed in the periphery of the lesion. From day 3, it moved toward the center of the cryolesion with repulsion of the necrotic area of 1 millimeter each day. The area of necrotic tissue collapsed and from day 7 to day 15 an organization process could be seen in the former necrotic area. An important bile duct proliferation was observed (Fig. 4). At day 20, half of the formerly necrotic tissue had vanished and was replaced by fibrotic tissue. At day 32, an acellular scar, 18 mm in diameter and 1 mm thick was found on the liver surface.

DISCUSSION

We have shown the feasibility of cryosurgery for a large volume of hepatic tissue with no morbidity and no sequelae. The 20-min freezing process was well tolerated in all animals, and no hypothermia occurred during the procedure. Hepatic cryolesions obtained after contact cryosurgery were homogeneous and reproducible. They underwent total necrosis by days 2 and 3, followed by inflammatory cell migration from the margin of the lesions and tissue regeneration with bile duct proliferation. At day 32, only fibrotic scars remained. Similar results were previously obtained with a single liver cryolesion in dogs [14]. It was also shown that major blood vessels were remarkably resistant to cold injury, and no late sequelae such as hemorrhage, thrombosis, or vascular dilation were seen [15]. It is well established that cryosurgery can be effective in the treatment of experimental liver tumors [16,17]. Jacob et al. [18] showed significantly increased survival in rats undergoing cryosurgery rather than surgical resection or tumor infarction. Similarly, it has been shown that the clinical use of cryosurgery was feasible and effective for treatment of liver tumors and investigators have proposed cryosurgery as a treatment for unresectable hepatic tumors [13,19]. Ravikumar et al.

[20] achieved a 62% 5-year overall survival and a 24% 5-year disease-free survival in a series of 32 patients treated with cryosurgery for unresectable primary or metastatic liver tumors.

The effects produced by the use of cryosurgery have a unique quality distinguishable from other means of producing necrosis. The tissue response is so characteristic that it is called cryonecrosis [21]. It begins during the thawing period with a considerable amount of edema and reddening. During the next hours, vascular stasis, thrombosis, and ischemia occur. The lesion is complete between day 2 and day 3. Histologically it appears to be an ischemic infarct.

One of the major problems limiting the more widespread use of cryosurgery is the difficulty of controlling the amount of tissue that is destroyed during treatment. Cryonecrosis occurs inside the frozen region of the liver. No satisfactory technique has been developed so far to precisely assess its size. As the extent of cryonecrosis is directly dependent on the accurate knowledge of the cryolesion (iceball) size, it seems that the control of the cryolesion growth using some form of iceball monitoring is necessary to ensure precise control of cryosurgery. Visual monitoring is only possible for surface lesions; even in that situation, there is an overestimation of the lateral spread of freeze that increases the risk of undertreatment and residual disease. In a controlled trial of the use of visual monitoring, it has been shown that the maximum error for lateral spread of freeze for centimetric cryolesions ranged between 5.5 and 12.5 mm [22]. Furthermore, the depth of the iceball can only be estimated by extrapolating its radius as seen on the surface of the liver [23] and lesions that are not visible or palpable cannot be treated by using visual monitoring.

Previous experimental and clinical studies have shown that the freezing process can be visualized by sonography [24–26]. It is a reliable, simple, noninvasive technique of controlling the iceball boundary. Our study confirmed that the solidification interface can easily be observed and monitored by continuous intraoperative ultrasound. Comparison of the necrotic zone size at days 2 and 3 and iceball size after a 5-min freezing cycle revealed remarkable correlation (17.7 ± 1.2 mm for intraoperative ultrasound monitoring versus 17 ± 0.3 mm for pathology results, mean radius ±SD). All the liver tissue located inside the hyperechoic rim turned necrotic.

Temperature monitoring is essential to achieve cryodestruction of tissue with surgical precision. Thermocouples and pyrometer systems afford a precise means of assessing the temperature and controlling the extent and depth of freezing [27]. Thermocouples are implanted near the outer border of the lesion to determine when the freezing boundary reaches a predetermined point. The major problem of this quantitative technique is the difficulty to place accurately the thermocouples, if the site is deep or not easily accessible. Another problem is to choose a threshold of temperature that is considered as lethal for cells and should be used routinely in cryosurgery. Although most animal tissues begin to freeze at about −2° to −3°C that does not mean total cellular destruction. In vitro studies such as freezing a suspension of normal and neoplastic cells, show substantial cell death at −20°C and total cellular death between −40°C and −50°C, but these data are difficult to extrapolate to cryosurgery in vivo [28]. In cryosurgical literature, the threshold of −20°C is commonly considered the optimal temperature at which to attain in treatment of small cutaneous tumors [29]. Gage [30] considers that a good and safe technique of cryosurgery requires a goal of −40°C in 5 millimeters of the normal tissue surrounding malignant tumors, which means temperatures of at least −50°C within the tumor and at its periphery. Such goals are difficult to achieve when treating large-volume liver tumors, i.e., those more than 4 cm in diameter. In our study, comparison of temperature curves and pathology results suggested that if −15°C could be achieved, there was a complete necrosis of normal liver parenchyma. Such a temperature should be obtained in the normal liver 5–10 mm away from the tumor to obtain safe cryoablation. Furthermore, by freezing both the tumor and the surrounding margin of normal liver, one may destroy the neoplastic lesion both directly by freezing and indirectly by depriving the tumor tissue of its vascular supply from the surrounding frozen liver tissue.

Another technique used to monitor the freezing process is based on low frequency bioelectric impedance measurement [31]. It is considered the most accurate method of monitoring cryonecrosis. When all the bound water is frozen and all its electrolytes are crystallized out, there is cessation of flow of electrical current within the cell because resistance to electrical flow is then complete. In cryosurgery, impedance is measured between an electrode implanted near the outer border of the lesion and a reference electrode implanted at a distance. When the solidification interface reaches the electrode needle there is a sudden increase in tissue resistance. Threshold values of 1,000–2,000 kΩ are usually used to monitor cryosurgery with impedancemetry.

In our experiment the use of impedancemetry to monitor cryonecrosis turned out to be difficult. Measures were hardly reproducible and it appeared very difficult to reach the 1,000-kΩ threshold. Comparison of impedance curves and pathology results showed that a complete necrosis could be achieved at 17 mm from the axis of the cryoprobe even though the 1,000-kΩ threshold was not reaching during freezing. We conclude that impedancemetry is too difficult to use and not precise enough for monitoring of a large volume of liver cryosurgery.

In the clinical setting, liver metastases are rarely on the liver surface. It is therefore frequently necessary to

use large trocar probes injected into the liver parenchyma. This situation is somewhat more complex than the one we chose because the proximity of large liver vessels may make freezing more difficult. In our study, we have deliberately used a technique of contact cryosurgery to obtain reproducible and comparable cryolesions. This was the easiest way to make precise and comparable measurements.

CONCLUSION

Modern cryosurgery with liquid nitrogen is a safe and relatively simple procedure. The monitoring of cryolesion growth with intraoperative sonography and thermometry allows precise control of cryosurgery and correct evaluation of the real extent of liver cryonecrosis. In normal liver, the solidification interface visualized with intraoperative ultrasound correspond to a −15°C temperature. In our study, complete liver necrosis was achieved for all the liver tissue located inside this solidification interface.

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